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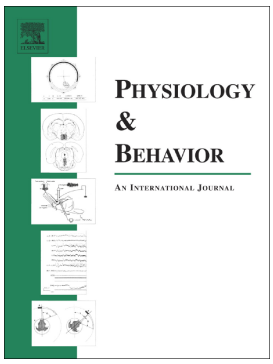

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Establishment of a Protocol for the Isolation of Feline Pancreatic Islets

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Abstract

Diabetes mellitus is a common endocrinopathy in cats that is associated with pancreatic islets lesions. Research on isolated islets contributed to the understanding of the pathophysiology of human diabetes. Therefore, by improving the existing methods of isolation in cats, we aimed at increasing islet yield, purity and viability of feline isolated islets.

Islet isolation was accomplished by pancreas perfusion with 80 ml of Collagenase type IV through the pancreatic duct at the site of the major papilla. The enzymatic digestion was combined with mechanical disruption and controlled by dithizone staining. Purification was performed by filtration and handpicking. Purified islets were plated on extracellular matrix pre-coated plates and cultured for 48h.

Feline islets with a high degree of viability and purity were isolated and cultured for the first time. Although the percentage of islet free from the acinar tissue relative to the total number of isolated islets was low compared to other species, the suggested protocol represents a promising progress in the procedure of islet isolation in cats.

Keywords: Pancreatic Islets, feline diabetes,

Introduction

The increasing prevalence of diabetes mellitus in cats in the past years [8] has generated the necessity of studying the pathophysiology and therapeutic strategies in this species. The reason for the progressive reduction in functional beta cell mass is still unknown and requires further investigation. Different hypotheses include the compromised beta cell identity subsequent to chronic hyperglycemia and glucotoxicity [10]. Which of these factors is of primary importance in diabetic cats and which of these may be crucial to the progression of the disease still needs to be defined [2,5].

Isolated islets in humans and rodents serve as in vitro models for the understanding of the mechanisms of beta-cell death and its prevention but also for antidiabetic drug development and screening [7]. However, such studies were hampered in cats because of the lack of a successful method for the isolation of pancreatic islets in this species. Major conclusions from the few previous studies [6 1 12,13] were that the paucity of collagen in the feline pancreas may be responsible for the difficulty of obtaining pure islets free of acinar cells. In general, islet purity, quantity and quality were not comparable to those in other species, in particular in rodents [9].

Material and Methods

A method currently used in mice [4] was adapted to the specific characteristics of the cat pancreas and tested in a male neutered cat that was euthanized at the Clinic for Small Animal Internal Medicine, University of Zurich (Switzerland) because of an end-stage lymphoma. After the owner has granted the permission to perform the necroscopy and to procure tissue for research purposes, the clinically dead cat was placed in dorsal recumbency, the hair surrounding the incision site were removed by clipping and the skin was aseptically cleaned. A ventral midline laparotomy was performed and the pancreas

was localized and chilled with NaCl 0,9% at 4-8°C (Fresenius Kabi AG, Bad Homburg, Germany). Based on the results from a pilot study which assessed the best-suited perfusion method in cats (data not shown), the pancreatic duct was cannulated (25G Venofix Safety system; Braun, Melsungen, Germany) at the major papilla after clamping the common biliary duct, duodenum and pylorus. The pancreas was perfused with 80 ml of an enzymatic solution containing 50 mg/ml of collagenase type IV (Worthington, NJ, USA), 10 mg/ml of DNase I (>2000 U/mg, Roche, Germany) and 1% Hepes (1M) in HBSS (GIBCO, Paisley, Scotland). A minimum of 80 ml solution was needed to achieve a distension of the pancreas of approximately 90%. After the entire pancreas was greatly distended, a total pancreatectomy was performed, the excised pancreas was immersed in a 500-mL sterile beaker with 50 ml ice-cold enzymatic solution (4°C) of the same composition and transported on ice to the laboratory for immediate processing.

The pancreas was digested in a water-bath at 37 °C for 80 min with manual shaking at 5 min intervals. When the percentage of free islets, assessed by staining with dithizone (DTZ); 50 mg DTZ powder was reconstituted with 10ml of Dimethyl sulfoxide (DMSO, Sigma-Aldrich, Saint Louis, USA) and 30ml of phosphate-buffer saline (PBS); at 5 min intervals, reached approximately 50%, the digestion was stopped by the transfer of the pancreas to 4°C.

At the end of the digestion step, the suspension was manually shaken 30-40 times, diluted with 35 ml of ice cold quenching buffer (HBSS with 2,4% HEPES 1M and 0,5% BSA) and centrifuged at 550 x g for 2 minutes at 4°C. The recovered pellet was re-suspended in 20 ml of ice-cold quenching buffer (HBSS with 2,4% HEPES 1M and 0,5% BSA), tube was inverted 3 times, and centrifuged again under the same conditions described above.

The pellet was re-suspended in ice cold quenching buffer and filtered through a 500 µm strainer (136 cm, SEFAR PETEX, Heiden, Switzerland) using a 16G/10 cm metallic needle (Delvo, Biel, Switzerland). After resuspension in fresh culture medium RPMI-1640 (11% fetal calf serum, 1,14% streptomycin-penicillin, 0,11% gentamycin, 0,11% fungison, 1,14% Glutamax and 11mM of glucose; Gibco, Paisley, Scotland), DTZ-positive islets were hand-picked and cultured in fresh culture medium at 37°C/5% CO2 for 24 h to recover from the stress of the isolation.

3.6 Islet quality

Quality (morphology and purity; defined as the percentage of free versus trapped islets to the total number of islets counted) and the size of islet preparations was assessed 24 h after isolation by staining 3.5 ml aliquots of purified islet solution with DTZ. An eyepiece with a 1 mm scale (10 µm grid; E-PI 10x/20, Zeiss, Feldbach, Switzerland) was used to determine the islet size.

3.7 Islet viability

After 24 h in culture, purified islets were handpicked and placed into 3.5 cm petri dishes (Corning, USA) containing 3 ml of culture media.

Islet viability (n=20) was assessed by double fluorescence staining with 20 µl propidium iodide (PI 1:10) followed by 20 µl of fluorescein diacetate (FDA; 1:100; Life Technologies, Foster city, USA) in 3 ml culture media for 30 seconds and then washed in PBS. The average viability of each islet was assessed with inverted wide field fluorescence microscope (Axiovert 200M, Zeiss, Feldbach, Switzerland) on five separate fields based on the percentage of viable versus dead cells.

3.8 Islet culture

Free islets (8 wells; 15 islets/well) were cultured in 24-well ECM-coated plates (Novamed Ltd, Jerusalem, Israel) in 500 µl of RPMI-1640 medium at 37°C/5% CO2 for 48 h. Islet survival under culture was measured as the percentage of attached islets, which were not washed off during medium changes, to the total number of plated islets.

Results

Warm ischemia time (time from death to placement of the pancreas on ice) was of 35 minutes. After 80 min of digestion, approximately 50% of the DTZ-positive cells clusters were islets free of exocrine tissue. Two hundred of these free islets were handpicked for the assessment of their morphology, size, viability and to be cultured (Fig.1). The diameter of 72% of the islets was between 50 to100 µm, while it was smaller than 50 µm in the remaining islets. Islet integrity and shape were preserved 24 h after the isolation. Islet viability determined by FDA/PI after the isolation was of 75% (Fig.2). Under culture, islet adhesion to ECM plates was observed at the earliest after 24 h of incubation and increased over time, with an average islet adhesion to the plate of 97% after 48 h.

Discussion

In situ cannulation of the pancreatic duct through the major papilla is difficult in cats [12], however, it enabled a more efficacious diffusion of our enzymatic solution into pancreatic acini compared to other methods [12].

The long digestion of the pancreas resulted in islets of high purity although the total number of free islets was low. Most isolated islets had a diameter smaller than 100 µm, which might be associated with islet fragmentation [11]. Considering these findings, enzyme blends of higher purity and more precise composition may be more appropriate to ensure consistent degradation activity without deteriorating islet integrity [3].

While mouse islets are easily identified [3], freshly isolated cat islets appeared as dark yellow spheroids with irregular edges that are not easily distinguishable from aggregates of exocrine acinar cells. Therefore, handpicking after beta-cell specific staining was essential for the evaluation of their morphology and purity.

Islet viability after the isolation was satisfactorily high compared to that measured by others [1,6]. Islet survival under culture conditions was indirectly measured by assessing the adhesion rate of islets to the culture plate and revealed to be increased by the plating on ECM compared to uncoated plates. Forty-eight hours after the plating, glucose-stimulated insulin secretion (GSIS) was performed with the isolated islets as previously described in mice [3]. Unfortunately, islet adhesion revealed to be insufficient and most of the islets were removed during the several washing steps required by the GSIS test. Because the functional potency of purified islets is measured by calculating their secretion index as the ratio of stimulated to basal insulin secretion normalized by the insulin content, no firm conclusions could be drawn regarding the functionality of the isolated islets.

In conclusion, we were able to isolate and culture islets with a degree of viability and purity that was significantly higher compared to our and others' previous studies [12]. Feline islets could survive in culture up to three days under the conditions applied in this study, however, as the yield of free islets was low compared to data on other species [9], the isolation and culturing conditions used to generate these preliminary data will have to be further improved to obtain larger numbers of viable purified islets for in vitro studies.

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Figures

Figure 1: Islet preparation stained with dithizone. Pancreatic islets of cat.

On the day of isolation, DTZ-positive islets had an irregular shape and were free from acinar tissue. Dithizone staining. Magnification X10; Axiovert 40 CFI, ZEISS.

Figure 2: Islet viability assessment. Pancreatic islet of cat.

Viable islet stained green (PI) while dead cells stained red (FDA). FDA/PI fluorescent staining. Magnification: X200; Axiovert 200M, ZEISS.

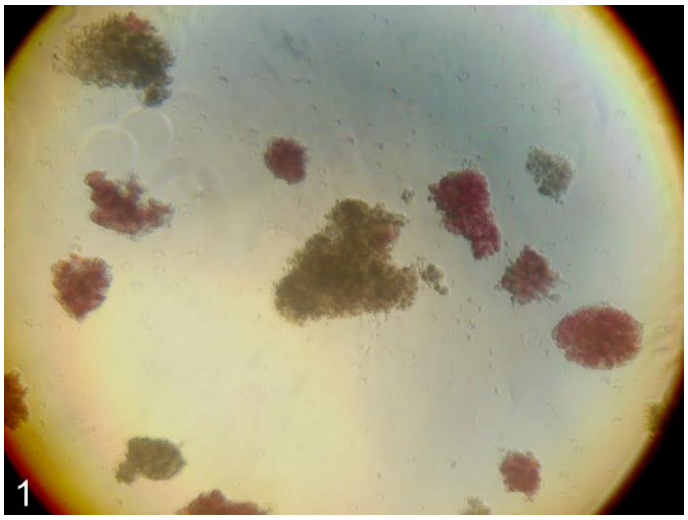

Figure. 1

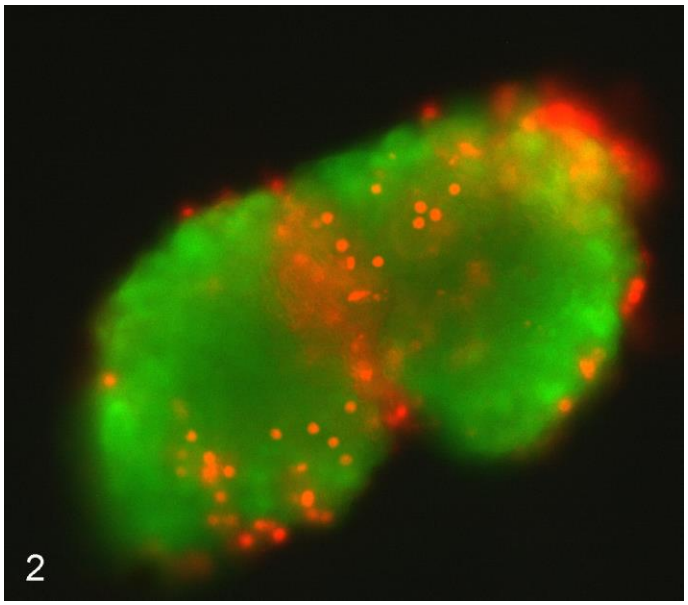

Figure. 2